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An improved method for recovering intact pulsed field gel purified DNA, of at least 1.6 megabases

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The widespread use of yeast artificial chromosomes (YAC's) as cloning vectors, has created a demand for a reliable method to recover intact chromosomal DNA from preparative pulsed field gels. DNA isolated in this way can be used in a variety of procedures including micro-injection into mammalian cells (1) and the creation of cosmid and phage sublibraries (2). Schedl *et al.* (1) describe a procedure in which YAC DNA from a preparative pulsed field gel slice is concentrated into 4% NuSieve and the intact DNA subsequently recovered by melting the agarose and digesting with Gelase. Here we describe a number of modifications to this method resulting in significant increases in the yield of DNA and speed of recovery. Intact DNA as large as 1.6 megabases was recovered from a pulsed field gel. This result was achieved primarily by improving the method for concentrating DNA and hence recovering it in a minimal volume of buffer. We also found that several other manipulations were crucial in establishing this method as a routine and reproducible procedure for recovering intact megabase sized DNA. Material of this size is particularly susceptible to nicking and degradation which were minimised by the scrupulous attention to sterility, care in reducing photodamage and shearing forces and maintaining the recovered DNA on ice.

Yeast DNA was prepared in agarose plugs (3) using standard procedures (4) and loaded on to a preparative pulsed field gel. We investigated several types of agarose and found that a high gel strength, low electroendosmosis (EEO) pulsed field (PFGE) grade agarose, such as Boehringer MP, when used at a concentration of 0.5%, offered significant advantages over the 1% low melting temperature (LMT) agarose used by Schedl *et al.* Gels were run in a BioRad CHEF DR-III at 6 volts/cm., using either 60 second pulses for 20 hours (which resolved up to 1.2 megabases) or 80 second pulses for 24 hours (which provided optimal separation in the size range 1–1.8 megabases). The running buffer was 0.5×TBE (45 mM Tris, 45 mM Boric acid, 0.5 mM EDTA). We found it was essential to use autoclaved buffer both for casting and running the gel and rinsing out the electrophoresis tanks prior to use.

At the conclusion of electrophoresis, only the outer tracks of the gel are stained with ethidium bromide and photographed under UV light. Exposure of chromosomal DNA to ethidium bromide and even a brief dose of long wave UV light produces sufficient nicking to reduce the yield of recovered DNA significantly. The positions of the yeast bands to be recovered are marked on the stained gel segments, the gel reassembled and a sterile scalpel blade used to excise the bands from the unstained part of the gel.

Gel slices are transferred to a standard agarose gel casting tray and positioned parallel to the direction of the applied electric field. The slices are embedded in 4% FMC NuSieve GTG agarose, in sterile 0.5×TBE, and the gel run at 2.4 volts/cm. for 16 hours. During the run, DNA migrates from the 6.5 cm. long gel slice into the NuSieve and is concentrated into a small volume beyond the end of the slice. Using a PFGE grade agarose for the initial preparative gel offers two significant advantages. Firstly, because migration in a 0.5% Boehringer MP agarose gel is 2.5 times faster than in a 1% LMT agarose gel, the run time is considerably reduced. Secondly, elution of the DNA from the gel slice into the NuSieve is more rapid than from LMT agarose so that the DNA is concentrated into a smaller volume of agarose. This can critically affect the subsequent recovery of DNA as a higher concentration at this stage yields a greater quantity of intact DNA. Once again, the DNA must not be exposed to ethidium bromide or UV light at this stage. Elution can be monitored by including

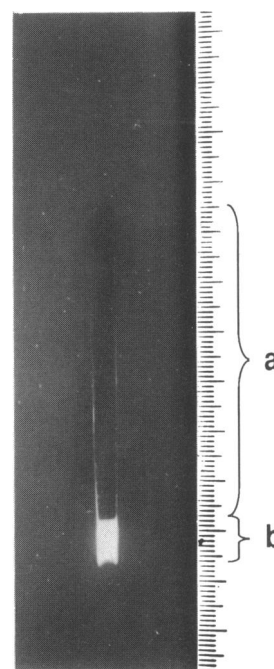


Figure 1. 1100 Kb yeast chromosomal DNA concentrated in 4% NuSieve agarose. 'a' — gel slice, 'b' — concentrated DNA. See text for experimental details.

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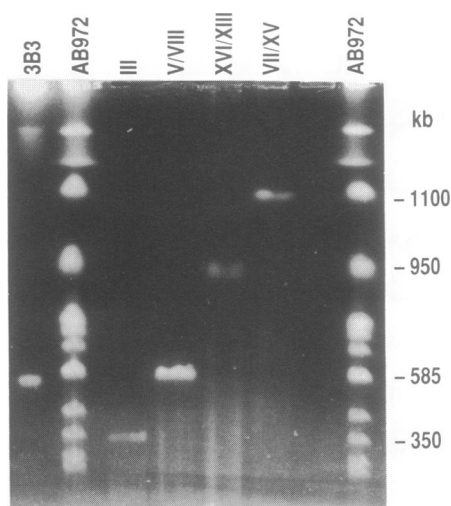


Figure 2. 50 μ l aliquots of yeast chromosomal DNA (from AB972) run on a 1% agarose gel at 6 volts/cm, using 90 sec pulses for 36 hours. 3B3 is a *Schizosaccharomyces pombe* strain carrying a 550 Kb minichromosome.

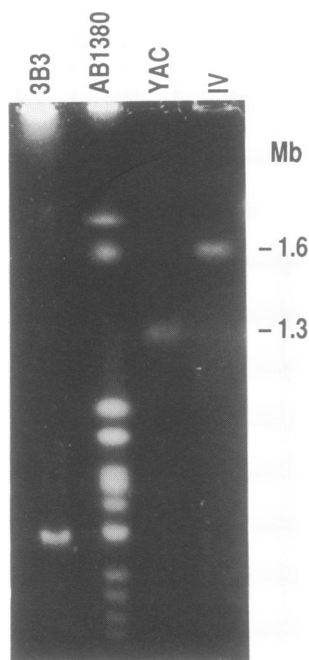


Figure 3. 50 μ l aliquots of YAC and yeast chromosomal DNA (from AB1380) run on a 0.5% Boehringer MP agarose gel at 6 volts/cm, using 80 sec pulses for 20 hours.

a dummy gel slice, for staining and photography, from which DNA is not required (fig. 1).

The concentrated DNA is excised from the NuSieve agarose and equilibrated for 2 hours in 100 times the volume of a buffer containing 30 mM NaCl. The choice of buffer is determined by the next manipulation to which the DNA will be subjected following recovery from the agarose. We can confirm already published data that polyamines are not required (5). The agarose

blocks are heated at 68°C for 5 min. to melt the agarose and then allowed to cool to 40°C. Two units of β -agarase 1 (New England Biolabs) are added to each tube and incubated at 40°C for 2 hours. We have found that β -agarase 1 not only digests agarose in the buffer recommended by the manufacturer, but also in any of the five Boehringer Mannheim restriction enzyme buffers, Boehringer Mannheim T4 DNA ligase buffer or KGB buffer (6). Successful agarase treatment is checked by incubating the DNA on ice for 10 min and then pooling the aliquots by slowly pipetting using a cut-off plastic tip. No lumps of undigested agarose should be present. An aliquot of the recovered DNA is run out on a pulsed field gel to check integrity and concentration. Either 1% standard agarose or (for speed) 0.5% Boehringer MP can be used at this stage. DNA is slowly transferred from the tube to the gel using a cut-off plastic tip and sealed into the slot by slowly dripping on semi-molten 1% LMT agarose. Figure 2 shows the recovery of intact DNA from yeast chromosomes III (350 Kb), V/VII (585 Kb), XVI/XIII (950 Kb) and VIII/XV (1100 Kb), whilst figure 3 shows DNA derived from a 1.3 Mb. YAC recombinant and from yeast chromosome IV (1.6 Mb). DNA extracted by this method should be used as soon as possible, but can be stored on ice for several days without significant degradation.

Using this method we have routinely recovered high quality intact YAC DNA for cosmid construction (manuscript in preparation). By using a PFGE grade agarose for the initial gel, the DNA is concentrated into a small volume (about half that achieved by using LMT agarose). This volume reduction offers important advantages if the DNA is to be used in a subsequent ligation reaction. Furthermore, the choice of β -agarase 1, which is active in a wide variety of buffers, can provide recovered DNA in a buffer suitable for subsequent manipulations.

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